

The Office Action correctly points out that Sisler does not teach or suggest the use of 1-MCP under reduced pressure. All of the disclosure of Sisler teaches the use at normal pressures.

Contrary to what the Office Action states, Huang does not disclose, teach, or suggest pretreatment of flowers with 1-MCP at reduced pressure. The Huang abstract states:

“It was found that when flowers are treated prior to transport by the low-pressure fumigation method developed by the Bureau of Animal and Plant Health Inspection and Quarantine (e.g., fumigation for three hours at low pressure (250 torr) with a mixture of methyl bromide (10 g/m^3) and phosphine (3 g/m^3), adverse effects on quality and vase life following transport of *Oncidium* cut flowers were minor compared to those seen in high-dose fumigation with methyl bromide (24.5 g/m^3), and these adverse effects could be effectively alleviated by pretreatment with ‘TARI’s Onc 1’ and 1-MCP.”

Page 4 of Huang, section number 2 discloses that the orchid flowers are first treated with 1-methylcyclopropene under ambient conditions and then fumigated at reduced pressure. (see translation page 4, lines 7-13). In most of the examples there is a simulated transportation step between the 1-MCP treatment and fumigation. Each of the disclosures in Tables 3, 4, 5, and page 12 of Huang refer only to the fact that low pressure fumigation is done with methyl bromide or phosphine. The general procedure in section 2 of Huang describes how the cut flowers are treated with TARI's Onc 1 and 1-MCP as a separate step from the low pressure fumigation with methyl bromide or phosphine. There is no disclosure, teaching, or suggestion in Huang alone, or in combination with Sisler, that plants are, or should be, treated with 1-MCP at reduced pressures. Tables 3, 4, and 5, and page 12 of Huang do not teach the use of 1-MCP at reduced pressure, only that the separate step of fumigation was conducted at reduced pressure. Thus, the reference does not disclose Applicants' claimed invention; the treatment of plants and plant parts with 1-methylcyclopropene at reduced pressure and the advantages of such treatment. The Office Action states that since the prior art teaches low pressure that it would have been obvious to use low pressure for the treatment with 1-MCP alone. However, this assumption misses the purpose of utilizing low pressure for fumigation. Huang indicates that fumigants themselves cause damage to the plants. (See page 11, lines 8-26, particularly lines 22-26). Thus, the purpose of the use of low pressure in the fumigation step is to reduce the dose of fumigant while still

maintaining activity against the insect pests. (See page 12, lines 6-19). 1-MCP, on the other hand, does not damage plants. In fact, it is known to preserve plants. As a result, there is no motivation to use 1-MCP at a reduced dose as there is with using the fumigants. It is clear from Huang that the fumigation and 1-MCP steps in the process are carried out entirely separately. If it was obvious to carry these steps out simultaneously, Huang would have done so in order to reduce the number of steps involved in the process. If anything, the Huang reference teaches away from the use of 1-MCP at low pressures.

Rossi merely describes a container for materials which allows for the evacuation of air after closing the container. The purpose is to protect the contents of the container, be they foodstuffs, chemicals, pharmaceuticals, or any other materials requiring an air-free environment (see the abstract). For foodstuff, the purpose is to remove air which may destroy the freshness of the foodstuff (see col. 2, lines 40-48). There is no disclosure, teaching, or suggestion, either alone or in combination with Sisler and Huang, that such an apparatus would be useful to treat a foodstuff or an agricultural product with a gas such as, for example, 1-MCP.

The Office action states that in the absence of any criticality and/or unexpected results, the instant invention is considered obvious to one skilled in the art at the time of invention. However, Applicants' Examples clearly demonstrate the unexpected advantages of the reduced pressure treatment with 1-MCP over the standard treatment. One distinct advantage is in the significantly reduced treatment times.

Therefore, one of ordinary skill in the art at the time of the invention would not have found Applicants' invention obvious in light of Sisler, Huang, and Rossi.

Rejection under 35 USC §103(a) - 2nd Rejection

Claims 1-5 are rejected under 35 USC §103(a) as being unpatentable over Zanella, *Postharvest Biology and Technology* 27 (2003) 69-78 ("Zanella"). The Office action states that Zanella teaches that, at low-pressure, treatment with 1-MCP has better control of superficial scald and ripening.

There are two issues related to this rejection. The first concerns the point in Zanella's process in which the 1-MCP treatment occurs. Zanella, page 71, Section 2. Material and methods describes the process as first treating the fruit with 1-MCP (Section 2, lines 4-13), then keeping the fruit at 2.5 °C and ~95% relative humidity for 7 days, then storing in either normal or

controlled atmosphere conditions. There is no disclosure, teaching, or suggestion of treating the fruit with 1-MCP at reduced pressures.

The second issue relates to controlled atmosphere storage itself. Controlled atmosphere storage is a well-known technique in which the temperature, oxygen, carbon dioxide, and humidity levels are carefully controlled in order to preserve the freshness of produce. The oxygen levels are typically reduced by displacement with nitrogen. The attached references (Washington Apple Commission, especially paragraph 6; and Kupferman, *Controlled Atmosphere Storage of Apples and Pears*, Washington State University - Tree fruit Research and Extension Center, December 2001) each describe the effects and the use of controlled atmosphere storage conditions on fruits. These references teach that the percentages of oxygen and carbon dioxide content in the atmosphere are controlled, that is, their partial pressures, not the total pressure of the controlled atmosphere.

Huang has been addressed in the first rejection. The same considerations apply to its use in combination with Zanella in the second rejection. One skilled in the art, therefore, would not have found Applicants' invention obvious in light of Zanella and Huang.

Again, Applicants refer to the unexpected advantages of the reduced pressure treatment with 1-MCP over the standard treatment that is found in the Examples in the Application.

With this response, Applicants believe that the rejections have been overcome and the claims are in condition for allowance. Should the Examiner have any suggestions which may put the Application in better condition for allowance, Applicants' attorney is willing to discuss any such suggestions either by phone or at the U. S. Patent and Trademark Office.

Respectfully submitted,



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FACTS

▶ Controlled Atmosphere Storage (CA)

Eating crisp, juicy Washington apples year-round is possible due to controlled atmosphere storage. Known simply as "CA" in the industry, controlled atmosphere storage involves careful control of temperature, oxygen, carbon dioxide and humidity.

CA storage got its start in England before World War II when farmers discovered their produce kept longer if stored in an airtight room. It was up to scientists to unravel the reasons for longer storage.

Apples take in oxygen and give off carbon dioxide as starches in the flesh change to sugar. In the sealed rooms, this respiratory process reduced the oxygen, thus slowing the ripening process.

CA storage has come a long way since then, and researchers in Washington state have been among the leaders in this technology. CA was first used in the United States in the 1960s and Washington now has the largest capacity of CA storage of any growing region in the world.

The large, airtight CA rooms vary in size from 10,000 boxes to 100,000 boxes, depending on the volume of apples produced by the apple shipper and his marketing strategies.

CA storage is a non-chemical process. Oxygen levels in the sealed rooms are reduced, usually by the infusion of nitrogen gas, from the approximate 21 percent in the air we breathe to 1 percent or 2 percent. Temperatures are kept at a constant 32 to 36 degrees Fahrenheit. Humidity is maintained at 95 percent and carbon dioxide levels are also controlled. Exact conditions in the rooms are set according to the apple variety. Researchers develop specific regimens for each variety to achieve the best quality. Computers help keep conditions constant.

Timing of harvest is critical to good storage results. Apples picked too early will not store well in CA nor will those that are past the proper maturity.

In mid-August, apple growers start testing the maturity of their apples to accurately predict when to harvest their crop to put in CA rooms so the apples are mature, but not too ripe. Firmness, skin color, seed color, sugar level and flesh chlorophyll are tested.

When the proper growing and harvesting techniques are used, many varieties of apples can store for 12 months or longer in CA. Most of these apples are shipped to market between January and September. Regular refrigerated storage is used for much of the fruit marketed in the fall and early winter months.



The CA rooms and CA operators are licensed and certified by the Washington State Department of Agriculture.

Washington law places requirements on the length of time apples must remain in CA conditions to qualify as CA-certified. Then state inspectors check every lot of fruit as the lot comes off the packing line to make sure the apples meet maturity requirements, the same requirements the U.S. Department of Agriculture uses for apples being exported. Only then will the box be stamped with the warehouse number and the "CA" symbol.

Apples meeting these standards must be shipped within two weeks or be reinspected to meet the same requirements. If they don't pass, the shipper must remove the CA designation from the box.

Red and Golden Delicious apples must also meet Washington state's strict standards for firmness and appearance. These standards apply to all apples shipped under Washington Fancy and Extra Fancy grades.

Washington has the highest concentration of CA storage of any growing region in the world.

Eastern Washington, where most of Washington's apples are grown, has enough warehouse storage for 181 million boxes of fruit, according to a report done in 1997 by managers for the Washington State Department of Agriculture Plant Services Division. The storage capacity study shows that 67 percent of that space — enough for 121,008,000 boxes of apples — is CA storage.

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CONTROLLED ATMOSPHERE STORAGE OF APPLES AND PEARS

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INTRODUCTION

Controlled atmosphere (CA) storage recommendations for apples and pears have been surveyed on a worldwide basis since 1985 and have appeared as part of the proceedings for each International Controlled Atmosphere Research Conference.

This information was obtained by asking recipients of several e-mail mailing lists for input. The mailing lists used were the International Postharvest Mailing List (New Zealand), USDA Regional Project: NE 103 Postharvest Physiology of Fruits (USA and Canadian scientists) and the European Fruit Quality Group. Respondents were asked to submit information via a web site established for this purpose. Unfortunately, even following several requests for information, few scientists submitted information. I appreciate the information supplied by Ann Schenk (Belgium), Duncan Park and Tony Brown (New Zealand), Alex van Schaik (Netherlands), Kobus van der Merwe (South Africa), Paolo Bertolini (Italy), Robert Prange (Canada), Felix Lippert (Germany), Randy Beaudry, Elizabeth Mitcham, Bob Saffner and John Fellman (USA).

For each variety the survey included questions in five major areas:

- Range of atmosphere used
- Optimum levels of oxygen, carbon dioxide and temperature
- Scald susceptibility and control method
- Disorders brought on by low oxygen or high carbon dioxide
- Whether modified or CA shipping containers have been utilized.

Fresh Apple Consumption

There are over 100 varieties of apples grown commercially in the USA, but 15 varieties accounted for over 90% of the production in 1999. The top 5 varieties for the USA and Europe are similar:

USA	Europe
1. Red Delicious	1. Golden Delicious
2. Golden Delicious	2. Red Delicious
3. Fuji	3. Jonagold
4. Granny Smith	4. Granny Smith
5. Gala and Royal Gala	5. Gala

The total fresh USA production for the 2000 crop was 142 million (20-kg) units. In 2000, 33 million units were exported from the U.S. and 9 million units were imported. The top producing states were Washington, New York, Michigan, California, Pennsylvania and Virginia.

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In 1999, the average U.S. consumer consumed 8.7 kg of fresh apples and 9.7 kg of processed apples. On a national basis, 56% of the U.S.-produced crop was eaten as fresh fruit. Consumption of fresh apples in the U.S. has been declining since 1989, when consumption was 9.6 kg per person.

Is it possible that our postharvest methods of preservation have not met consumer expectations? Is enough attention paid to edible components of quality as we develop our CA recommendations? Washington state, whose growers have imposed the strictest grade standards in the United States, does not have shipping standards based on soluble solids or acid content, only on firmness and appearance.

Fresh Pear Consumption

The top 5 major pear varieties in the USA and Europe are:

USA	Europe
1. Anjou	1. Conference
2. Bartlett (Williams)	2. Williams
3. Bosc	3. Abate Fetel
4. Comice	4. Blanquilla
5. Seckel	5. Comice

OPTIMUM LEVELS OF CA ATMOSPHERES

Respondents provided information on the optimum levels for CA storage on the numerous varieties of apples (Table 1) and pears (Table 2). There is a range of optimum levels for each variety listed partly as a result of the intended marketing program in the region, regional climatic differences, different strains of a particular variety and the current level of technology available within a region. So, optimum levels for a particular variety are not necessarily the result of research trials established to determine how long a variety can be stored, rather the result of horticultural, technical and marketing constraints.

ATMOSPHERIC INDUCED DISORDERS

Low oxygen injury: The respondents generally agreed that low oxygen injury to apples appears first as a loss of flavor followed by an alcoholic flavor generated by anaerobic fermentation. In some varieties the red area of the skin will turn purple and green areas will bronze. In some rare cases there will be flesh browning.

Pears manifest low oxygen injury in much the same way as apples—through anaerobic fermentation.

High carbon dioxide injury: The skin of the fruit will be rough and stained with a snowflake pattern on some varieties. The flesh of affected fruit will brown (internal browning) and in many cases will develop cavities. The core tissue may brown (coreflush) or develop lens-shaped pits.

Pears subjected to high carbon dioxide develop flesh browning and cavities.

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COMMERCIAL STORAGE RISKS

According to the responses from the survey, most varieties of apples have similar storage risks. Storage scald is the most common risk on most apple varieties, especially Cortland, Empire, Granny Smith, Jonagold, Pacific Rose, Pink Lady, Red Delicious, Rome and Stayman.

Internal browning disorders are common to Braeburn, Empire, Fuji, McIntosh and Spartan.

Bitterpit is common to Cox's Orange Pippin, Golden Delicious, Jonagold and Northern Spy.

Short storage life is common to Gala, Elstar, Cox's Orange Pippin and Gravenstein. Firmness loss in storage is common to Elstar, Gala, Jonagold and Stayman, making these varieties also candidates for only short-term storage. Flavor loss in storage should also shorten storage life. Flavor loss is reported in Fuji, Gala, Golden Delicious, and Red Delicious.

In pears storage life is usually shortened by internal browning, skin yellowing or storage scald (Table 3).

In apples, the methods of controlling storage scald are the application of diphenylamine (DPA), storage in low oxygen (0.7%), delaying harvest, or initial low-oxygen shock. In pears, initial low oxygen (0.7%), ethoxyquin drench, or ethoxyquin imbedded in the paper wrapper are utilized to control scald.

SHIPMENT IN CA OR MA CONTAINERS

Several apple and pear varieties have been shipped in CA containers from the Southern Hemisphere. Apples shipped in CA containers include Braeburn, Cox's Orange Pippin, Royal Gala, Golden Delicious, Jonagold and Pacific Rose. Beurre Bosc and Williams pears have been shipped from South Africa in CA containers.

Modified atmosphere containers have been used to ship Cox's Orange Pippin, Royal Gala, Golden Delicious apples and Williams pears.

EXPERIENCE WITH APPLES IN WASHINGTON STATE

It is my observation, based on both research results and industry experience with CA storage that apple varieties can be divided into two types: those that are tolerant of high CO₂ and those that are not. Obviously, this is a broad generalization.

Carbon Dioxide Tolerant

Gala and Golden Delicious are varieties in the CO₂ tolerant category. These varieties also benefit from rapid reduction of atmosphere. Rapid CA is valuable in that it helps retain fruit firmness and acidity better than slowly established CA on these varieties. Thus, fruit at moderate temperatures can be placed into a low oxygen environment rapidly without problems.

How rapid is rapid? This question has taken on new meaning as currently available equipment can remove oxygen more rapidly than ever before. Ten years ago, 'rapid CA' was defined as having the first harvested fruit in a room down below 3% oxygen within 7 days. Now several rooms can be filled each day and the oxygen is below 3% within hours of harvest. Fruit should be cooled to below 15 °C prior to lowering the oxygen level.

Non-spur Golden Delicious apples should be harvested at 7.3 kg firmness with a starch rating of 2.7 (1 to 6 scale), soluble solids at 11.5%, and acidity of 0.7% if they are to be held in long-term

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CA. The rate of change of starch and firmness, as well as the absolute numbers should be considered.

Current conditions do not permit profitable marketing internationally of Golden Delicious with green skin color. Skin color on Golden Delicious is often a reflection of the amount of nitrogen in the tree. Nitrogen levels above 2.15% (leaf analysis) will result in soft Golden Delicious with very green color. If fruit is to be stored in CA, only fruit from trees with moderate nitrogen levels should be selected. There is a temptation to allow Golden Delicious apples to hang longer to promote the change in skin color; these fruit are not suitable for CA.

Gala and Golden Delicious can be stored as low as 1.0% oxygen with CO₂ levels up to 2.5% at 1 °C. As the temperature is lowered below this point, the oxygen should be raised. Regular storage should be at 0 °C.

Carbon Dioxide Intolerant

Fuji, Braeburn, Granny Smith and Rome are those varieties in the CO₂ intolerant category. The cells appear to be dense and air circulation around the cells within the fruit is difficult.

These apples should have the flesh temperature close to the storage temperature before the oxygen is reduced. These varieties have a tendency for internal browning (e.g., Braeburn browning disorder [BBD]), which is associated with the natural predisposition of the apple (and other preharvest factors) as well as the storage regime. These varieties may develop less internal browning when the fruit has been treated with DPA prior to storage.

Carbon dioxide should remain well below the oxygen level at all times with these varieties. Temperatures should be held slightly elevated during CA storage. For example, fruit stored at 1.5% oxygen should be stored with CO₂ below 0.5% at 1 °C, if the fruit is not overmature at harvest. It is not advisable to store waxed fruit in boxes with polyliners in CA as this can hinder air circulation within the fruit.

Storage in regular atmosphere can be at 0 to 1 °C. The fruit should be subjected to good air circulation and not be in polylined boxes or waxed.

Red Delicious

Red Delicious, Washington's dominant apple variety, is somewhat CO₂ tolerant and is also tolerant of rapid CA. However, producers have not seen the dramatic positive effects of very rapid CA on Red Delicious that are seen on Golden Delicious or Galas. Storage operators should realize that this fruit softens more rapidly in a bin than on the tree so CA should not be delayed after harvest.

Red Delicious should be harvested at 7.7 kg firmness with the soluble solids at 10.0%, acidity at 0.270%, with a starch rating of 1.6 (1 to 6 scale) for long-term CA storage. Usually these fruits are postclimacteric with ethylene concentrations of 5 to 6 ppm. The rate of change as well as the absolute numbers should be considered.

Storage scald susceptibility decreases with maturity and cold nighttime temperatures. Scientists have determined the risk of scald decreases as temperatures below 10 °C accumulate within 3 to 4 weeks prior to harvest. Once 100 degree-day units below 10 °C have accumulated, scald should not be a problem if Red Delicious are treated with DPA.

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Typical storage regimes for CA of non-watercored Red Delicious are 1.5% oxygen and up to 2.0% CO₂ at 0 to 1 °C. I have done trials using early season commercially harvested Red Delicious at 0.7% oxygen with good results. This low level of oxygen should never be used on watercored apples. Regular storage is usually at 0 °C or slightly below.

Pears

The dominant varieties of pears grown in Washington are Anjou, which is stored up to 9 months in CA, and Bartlett, which is stored up to 3 months in CA. Bartlett pears should be considered mature to harvest starting at firmness of 8.6 kg for CA storage and continuing to 7.7 kg for short-term storage. Anjou pears start at 6.8 kg firmness for CA storage and continue to 5.9 kg for short-term storage. Bosc pears start at 7.3 kg firmness for CA storage and continue to 6.4 kg for short-term storage. In all pears, it is important to monitor the starch level, because in certain seasons firmness will remain high for a time while the starch content changes. This starch change indicates a movement in maturity.

Pears must be at storage temperature before the oxygen is lowered. Anjou pears should be stored with the oxygen at least 1% higher than the carbon dioxide at all times, especially when the fruit is held at -0.5 to 1.0 °C. There has been some research that fruit stored at temperatures higher than 1 °C can tolerate higher CO₂ levels. In Steve Drake's (USDA-ARS) experiments with Anjou pears (1998 and 1999 crops), fruit stored with CO₂ levels 1% above the oxygen level had no internal browning, was greener, and had less skin marking than fruit stored at low CO₂ levels.

CA Storage Summary

In summary, for Washington state, CA regimes should take into account their effect on edible as well as visual quality. Successful storage in CA depends on the balance between temperature, oxygen and carbon dioxide. For example, at low levels of oxygen, more chilling damage may occur at higher temperatures. Many of the problems associated with fruit storage of certain varieties can be reduced when the fruit is near the storage temperature before the oxygen is reduced.

BEST AVAILABLE COPY**WASHINGTON STATE UNIVERSITY - TREE FRUIT RESEARCH AND EXTENSION CENTER****Table 1. Optimum levels for CA storage of apples.**

Cultivar—apples	Country*	Optimum O ₂ (%)	Optimum C'O ₂ (%)	Optimum temp (°C)	Storage life (months)
Belle de Boskoop	Netherlands	1.3	0.7	4.5 to 5	5.5
Braeburn	New Zealand	3	<1.0	0.5	6
	New Zealand	3	0.5	0.5	3 to 4
	South Africa	1.5	1.5	-0.5	8
	USA (WA)	1.5	0.5	0 to 1	10
Cortland (all strains)	Canada (NS)	1.5	1.5	3	8 to 10
	Canada (NS)	2.5	4.5	3	8 to 10
Cox's Orange Pippin	Netherlands	1.3	0.7	4	6.5
	New Zealand	2	<2.0	3.0	3
Elstar	Canada (NS)	2.5	4.5	0 to 0.5	no data
	Netherlands	1 to 1.2	2.5	1.8	7
Empire	Canada (NS)	2	0.5	2.0 to 2.5	8
	USA (MI)	1.5	<1.0	3.5	5 to 6
	New Zealand	2	<1.0	0.5	6
Fuji	USA (WA)	2.0	0.5	1	12
Gala	Canada (NS)	1.5	1.5	0 to 0.5	8
Royal Gala	New Zealand	2	2	0.5	4
	New Zealand	2	2	0.5	3 to 4
	South Africa	1.5	1.5	-0.5	7
	USA (WA)	2	1.5	0 to 1	7
Gloster	Canada (NS)	1.5	1.5	0 to 0.5	10
Golden Delicious	Belgium	2.5	1	0.5	9
	Canada (NS)	1.5	1.5	0 to 0.5	10
	Canada (NS)	2.5	4.5	0 to 0.5	10
	Italy	1	2	1 to 2	8 to 9
	Netherlands	1 to 1.2	4	1	8
	South Africa	1.5	2.5	-0.5	9
	USA (MD)	1.0	the lower the better	0	8 to 9
	USA (MI)	1.5	<3	0	6 to 8
	USA (WA)	2	1.5	0 to 1	9
Granny Smith	Italy	1	1	0	7 to 8
	New Zealand	2	2	0.5	6
	South Africa	1.5	1.5	-0.5 to +0.5	11
	USA (WA)	1.5	0.5	0 to 1	10
Gravenstein	Canada (NS)	1.5	1.5	3	4
Idared	Canada (NS)	2	0.5 to 1.5	0 to 3.0	10
	USA (MI)	1.5	<3	0	7 to 8
Jonagold	Canada (NS)	1.5	1.5	0 to 0.5	10
	Netherlands	1 to 1.2	4.5	1	9
Jonathan	USA (MI)	1.5	<3	0	5 to 6